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Major Article

Phlebotominae sand flies (Diptera: Psychodidae): potential vectors of American cutaneous leishmaniasis agents in the area associated with the Santo Antônio Hydroelectric System in Western Amazonian, Brazil

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ABSTRACT

Introduction: An entomological study was conducted as part of a vector-monitoring program in the area associated with the Santo Antônio hydroelectric system in State of Rondônia, Western Amazonian Brazil. **Methods:** Fourteen sampling sites were surveyed to obtain data on the potential vectors of *Leishmania* spp. in the area. Sand flies were collected from 2011 to 2014 during the months of January/February (rainy season), May/June (dry season), and September/October (intermediary season) using light traps arranged in three vertical strata (0.5, 1, and 20m). **Results***:* A total of 7,575 individuals belonging to 62 species/subspecies were collected. The five most frequently collected sand flies were *Psychodopygus davisi* (Root) (36.67%)*, Trichophoromyia ubiquitalis* (Mangabeira) (8.51%), *Nyssomyia umbratilis* (Ward & Fraiha) (6.14%), *Bichromomyia flaviscutellata* (Mangabeira) (5.74%), and *Psychodopygus complexus* (Mangabeira) (5.25%). These species have been implicated in the transmission of American cutaneous leishmaniasis agents in the Brazilian Amazon region and described as potential vectors of this disease in the study area. **Conclusions:** Additional surveillance is needed, especially in areas where these five species of sand fly are found.

Keywords: Vectors. Cutaneous leishmaniasis. Amazonian region.

INTRODUCTION

Phlebotominae sand flies (Diptera: Psychodidae: Phlebotominae) belong to a group of insects with public health relevance that are vectors of certain pathogens, including agents of leishmaniasis[1,2]. Currently, there are 274 recognized species of sand fly in Brazil, and approximately 10% of these species have been implicated in the transmission of American cutaneous leishmaniasis (ACL)[3,4]. Most of these species are endemic to the Brazilian Amazon region. The high biodiversity of the region may contribute to the variety of ecological systems that *Leishmania* Ross parasites and their respective mammalian reservoir hosts inhabit[5].

In the State of Rondônia in Western Amazonian Brazil, ACL is considered to be a major public health problem, with an annual incidence of 115 cases per 100,000 inhabitants. From 2011 to 2013, 2,945 confirmed autochthonous cases of the disease were reported[6]. In Rondônia, at least four *Leishmania* species have been associated with human infection, including *Leishmania* (*Viannia*) *braziliensis* Vianna*, Leishmania* (*Viannia*) *lainsoni* Silveira, Shaw, Braga & Ishikawa, *Leishmania* (*Viannia*) *guyanensis* Floch, and *Leishmania* (*Leishmania*) *amazonensis* Lainson & Shaw[7,8].

Previous studies have compiled interesting data on sand fly populations in State of Rondônia[9,10,11,12,13,14,15,16]. These studies and secondary data compiled in earlier checklists[17,18] identified 111 Phlebotominae species in State of Rondônia; at least 18 of these species have been associated with *Leishmania* infections[14].

As in other states of the Brazilian Amazon region, the territory of Rondônia contains infrastructure such as hydroelectric systems that impact the environment by suppressing the growth of vegetation. Because this human activity might influence the emergence of unknown epidemiological profiles of ACL, it is necessary to improve current knowledge of *Leishmania* species diversity and the temporal/spatial distribution of vector fauna in these areas using surveillance programs aimed at monitoring the transmission of disease. Thus, the aim of the present study was to provide data on the sand fly fauna of an area associated with a hydroelectric system in western Amazonian Brazil and identify potential vectors of ACL agents in this area.

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METHODS

Study area

Porto Velho (8° 45′ 43″ S, 63° 54′ 14″ W) is the capital of and largest city in the State of Rondônia. The average altitude ranges from 70 to 600m above sea level. The climate has been classified as tropical super humid (medium annual temperature 25.5°C), and the predominant vegetation is Amazonian tropical rain forest. This area is directly impacted by the construction of the Santo Antônio Hydroelectric System in the Madeira River. Porto Velho, which has an estimated population of 495,000 inhabitants, experienced 314 reported autochthonous cases of ACL in 2011, 2012, and 2013, accounting for more than 10% of the cases of ACL in the State of Rondônia[6]. Fourteen collection sites were selected as priority areas for a survey based on the recommendations of previous studies conducted as part of the Basic Environmental Plan (BEP) of the Santo Antônio Hydroelectric System (**Table 1**). These sites are located in the *terra firme* forest, which is characterized by distantly spaced apart trees (open canopy) with an average of 40m of canopy. Larger trees with a canopy of up to 55m such as *Castanha-do-Pará* (Lecythidaceae), *Muiaracatiara* (Anacardiaceae), *Tauari* (Lecythidaceae) and *Angelim* (Fabaceae) were also observed. The composition of palms (Arecaceae) is variegated in these dense forest environments.

Sampling

Before and after the dam was filled, eleven samples were collected between January 2011 and June 2014 during the rainy (January/February), dry (May/June), and intermediary (September/October) Amazonian seasons using light traps from the Center of Diseases Control (CDC) that were run from 06:00 p.m. to 06:00 a.m. for two consecutive nights. To ensure that the sampling effort was sufficient, a cumulative curve was constructed to calculate the Chao 1, Chao 2, Jackknife 1, and Jackknife 2 diversity indices using the data from all 121 sampled points. The sampling points comprised three traps positioned in different vertical strata, i.e., 0.5, 1, and 20m into the tree canopy, providing 72h of sampling time per collection site. Individual data on the vertical stratification were not available, and each collection site provided a single trapping sample. All collections were approved by the Brazilian Institute of Environment and Renewable Natural Resources (Authorization IBAMA n. 219/2013).

Sand fly processing

The collected individuals were identified according to their external morphological characteristics. Specimens that could not be identified in the field were stored in 70% alcohol, transported to the laboratory and mounted on glass slides with Berlese liquid (G.B.I. Laboratories, Manchester, England) using a modified version of the Young & Duncan[1] temporary mounting technique. The phylogenetic taxonomic criteria of Galati[19] were used to identify species, and generic abbreviations proposed by Marcondes[20] were used.

Acquisition of secondary data

Data on the average rainfall during the 2012-2013 period were obtained from the Porto Velho automatic station (National Institute of Meteorology, www.inmet.com.br).

TABLE 1- Collection sites used to catch sand flies in the area associated with the Santo Antônio Hydroelectric System.

	Coordinates		
Collection sites	South	West	Localities
P1	09°24'34.4"	064°44'39.2"	Sitio Samaúma I/Jacy Paraná
P2	09°25'09.2"	064°25'29.6"	Alto Rio Jacy Paraná
P3	09°15'45.2"	064°24'19.2"	Bairro Velha Jacy/Jacy Paraná
P4	09°15'02.5"	064°24'11.7"	Jacy Paraná/distrito
P5	09°05'41.3"	064°23'20.5"	Assentamento Joana D'Arc
P6	09°01'59.3"	064°08'54.7"	Reassentamento Morrinhos
P7	09°04'03.3"	064°10'49.3"	Reassentamento Santa Rita
P8	08° 51'55.8"	064° 03'38.3"	Reassentamento Vila Nova do Teotônio
P9	08°46'13.8"	064°04'41.2"	Vila Franciscana
P10	08°45'37.1"	064°01'42.4"	Sitio São Domingos
P11	08°48'36.8"	063°56'19.7"	Vila Santo Antônio
P12	08°45'45.0"	063°59'37.8"	Entorno do Canteiro
P13	08°46'21.0"	063°55'52.0"	Reassentamento Novo Engenho Velho
P14	08°35'01.5"	063°43'59.3"	Cujubim Grande

RESULTS

A total of 7,575 phlebotomine sand flies belonging to 64 taxa (62 species/subspecies) were collected over the course of 11,888h of sampling (**Table 2**). Fifteen genera were identified, including *Psychodopygus* Mangabeira (13 spp.), *Evandromyia* Mangabeira (10 spp.), *Psathyromyia* Barreto (8 spp.), *Trichophoromyia* Barreto (6 spp.), *Nyssomyia* Barreto (6 spp.), *Micropygomyia* Barreto (5 spp.), *Migonemyia* Galati (3 spp.), *Bichromomyia* Artemiev (2 spp.), *Sciopemyia* Barreto (2 spp.), *Viannamyia* Mangabeira (1941) (2 spp.), *Pressatia* Mangabeira (2 spp.), *Pintomyia* Costa Lima (1 spp.), *Lutzomyia* França (1 spp.), *Trichopygomyia* Barreto (1 spp.), and *Brumptomyia* França and Parrot (1 spp.).

The sampling sufficiency curve showed stabilization, reaching an asymptote for the four analyzed diversity indices (**Figure 1**). The presence of six species (*Trichophoromyia eurypyga* (Martins, Falcão & Silva), *Pressatia choti* (Floch & Abonnenc), *Sciopemyia fluviatilis* (Floch & Abonnenc), *Evandromyia pinottii* (Damasceno & Arouck), *Nyssomyia yuilli pajoti* (Abonnenc, Léger & Fauran), and *Trichophoromyia ruii*

TABLE 2 - Sand fly species from the eleven collection sites associated with the Santo Antônio Hydroelectric System in State of Rondônia, Western Amazonian Brazil, from 2011 to 2014.

	Species	Total	Percentage		Species	Total	Percentage
1	*Psychodopygus davisi*	2,778	36.67	35	*Evandromyia evandroi*	17	0.22
2	*Trichophoromyia ubiquitalis*	645	8.51	36	*Evandromyia walkeri*	17	0.22
3	*Nyssomyia umbratilis*	465	6.14	37	*Psathyromyia inflata*	16	0.21
4	*Bichromomyia faviscutellata*	435	5.74	38	*Micropygomyia longipenis*	16	0.21
5	*Psychodopygus complexus*	398	5.25	39	*Psychodopygus claustrei*	15	0.20
6	*Sciopemyia sordellii*	266	3.51	40	*Evandromyia monstruosa*	13	0.17
7	*Psychodopygus corossoniensis*	191	2.52	41	*Trichophoromyia brachipyga*	12	0.16
8	*Trichophoromyia castanheirai*	167	2.20	42	*Evandromyia bacula*	11	0.15
9	*Evandromyia saulensis*	152	2.01	43	*Psathyromyia dreisbachi*	11	0.15
10	*Nyssomyia antunesi*	149	1.97	44	*Evandromyia williamsi*	11	0.15
11	*Micropygomyia trinidadensis*	141	1.86	45	*Psathyromyia lutziana*	10	0.13
12	*Trichophoromyia eurypyga*	138	1.82	46	*Lutzomyia migonei*	10	0.13
13	*Psychodopygus chagasi*	138	1.82	47	*Bichromomyia olmeca nociva*	10	0.13
14	*Viannamyia furcata*	150	1.98	48	*Viannamyia tuberculata*	9	0.12
15	*Evandromyia infraspinosa*	120	1.58	49	*Evandromyia begonae*	11	0.14
16	*Nyssomyia yuilli yuilli*	102	1.35	50	*Evandromyia brachypalla*	8	0.11
17	*Psychodopygus hirsutus hirsutus*	90	1.19	51	*Lutzomyia carvalhoi*	8	0.11
18	*Pressatia choti*	89	1.17	52	*Psathyromia brasiliensis*	5	0.07
19	*Psychodopygus amazonensis*	76	1.00	53	*Psathyromyia* sp. (*shannoni* complex)	5	0.07
20	*Lutzomyia aragaoi*	73	0.96	54	*Psathyromyia dendrophila*	4	0.05
21	*Trichopygomyia trichopyga*	72	0.95	55	*Evandromyia pinottii*	4	0.05
22	*Psychodopygus carrerai carrerai*	65	0.86	56	*Pintomyia damascenoi*	3	0.04
23	*Psychodopygus leonidasdeanei*	55	0.73	57	*Pintomyia gruta*	2	0.03
24	*Psychodopygus paraensis*	51	0.67	58	*Micropygomyia micropyga*	2	0.03
25	*Psychodopygus geniculatus*	50	0.66	59	*Micropygomyia oswaldoi*	2	0.03
26	*Sciopemyia fluviatilis*	49	0.65	60	*Psychodopygus paraensis*	2	0.03
27	*Nyssomyia shawi*	43	0.57	61	*Psathyromyia scaffi*	2	0.03
28	*Nyssomyia anduzei*	32	0.42	62	*Nyssomyia yuilli pajoti*	2	0.03
29	*Micropygomyia rorotaensis*	32	0.42	63	*Psychodopygus* sp.	2	0.03
30	*Psychodopygus ayrozai*	32	0.42	64	*Brumptomyia travassosi*	1	0.01
31	*Trichophoromyia readyi*	23	0.30	65	*Trichopygomyia dasypodogeton*	1	0.01
32	*Psychodopygus squamiventris sensu latu*	27	0.35	66	*Psathyromyia triacantha*	1	0.01
33	*Psychodopygus lainsoni*	19	0.25	67	*Trichophoromyia ruii*	1	0.01
34	*Lutzomyia* sp.	18	0.24	**Total**		**7,575**	**100.00**

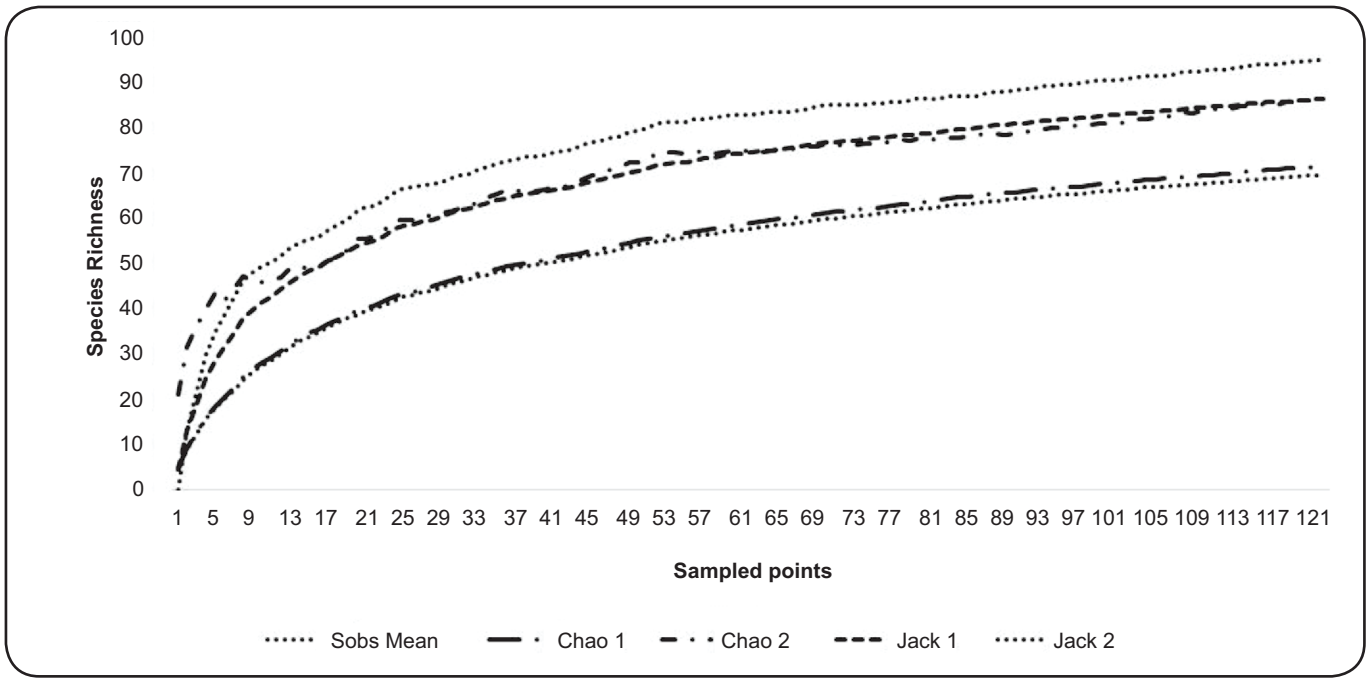

FIGURE 1 - Accumulation curve for sand fly species richness in 121 sites sampled from 2011 to 2014.

(Arias & Young) was documented for the first time in State of Rondônia.

As shown in **Figure 2**, an increasing number of sand flies was collected between the rainy and dry seasons (i.e., January/June), and the number of sand flies collected tended to decrease during the intermediary season (July/October). In 2012-2013, the first and second years after the dam was filled, the number of individuals collected increased during all of the collection seasons; thereafter, the number plateaued until June 2014.

The five most frequently collected species of sand flies have been implicated in the epidemiology of ACL in the Brazilian Amazonian region. These species included *Psychodopygus davisi* (Root) (36.7%), *Trichophoromyia ubiquitalis* (Mangabeira) (8.51%), *Nyssomyia umbratilis* (Ward & Fraiha) (6.14%), *Bicrhomomyia flaviscutellata* (Mangabeira) (5.74%), and *Psychodopygus complexus* (Mangabeira) (5.25%). The frequency with which each species was encountered in 2012 and 2013 was compared with the rainfall; the population size of these insects was found to increase at the beginning of the dry season (May/June) when pluviometric precipitation began to decrease (**Figure 3**).

DISCUSSION

A total of 118 species were identified in State of Rondônia, including the present six new records. Two previous studies conducted in this state had identified 22 new records[14] [16]. Therefore, it appears that new records of phlebotomine sand flies are continuously being discovered in Rondônia. The Chao/Jackknife indices calculated in the present study indicated that the sampling effort was sufficient to completely estimate the species richness in the studied region.

Several recent studies have described a high level of biodiversity of sand flies (>50 species registered in some of environments) in areas associated with hydroelectric systems in Brazil[21] [22] [23]. The present study is the first to provide a longitudinal evaluation of sand fly populations before and after the filling of a dam. The results of this study indicate that the filling of the dam may be associated with an immediate increase in the density of the sand fly population.

The proportion of sand fly populations that had been disturbed by environmental changes tended do decline over time, as evidenced by comparison with subsequent collections, suggesting an apparent reestablishment of the ecological system. The factors associated with these fluctuations have not been well defined. Environmental changes influence the biodiversity, population size, and proportion of dominant species of sand fly[24]. Long-term surveys are needed to evaluate whether sand fly populations are able to be replenished after a long period. A preliminary analysis found no correlation between the number of cases of ACL and variations in the number of flies (data not shown).

Psychodopygus davisi is a medically important species of sand fly. The anthropophilic behavior and widespread distribution of these insects in the Amazon basin[25] and previous records of natural *Leishmania* infections with this species suggest that this species is a potential vector of ACL agents in the studied region. This species has been shown to carry *L.* (*V.*) *braziliensis* and *L.* (*V.*) *naiffi* Lainson and Shaw in Serra dos Carajás[26] (Southern State of Pará, Brazil) and *L.* (*V.*) *braziliensis* in State of Rondônia[14] [27]. Recently, two females of this species from the area associated with the Belo Monte Hydroelectric System in State of Pará were found to carry peripylarian *Leishmania*-like flagellates (unpublished

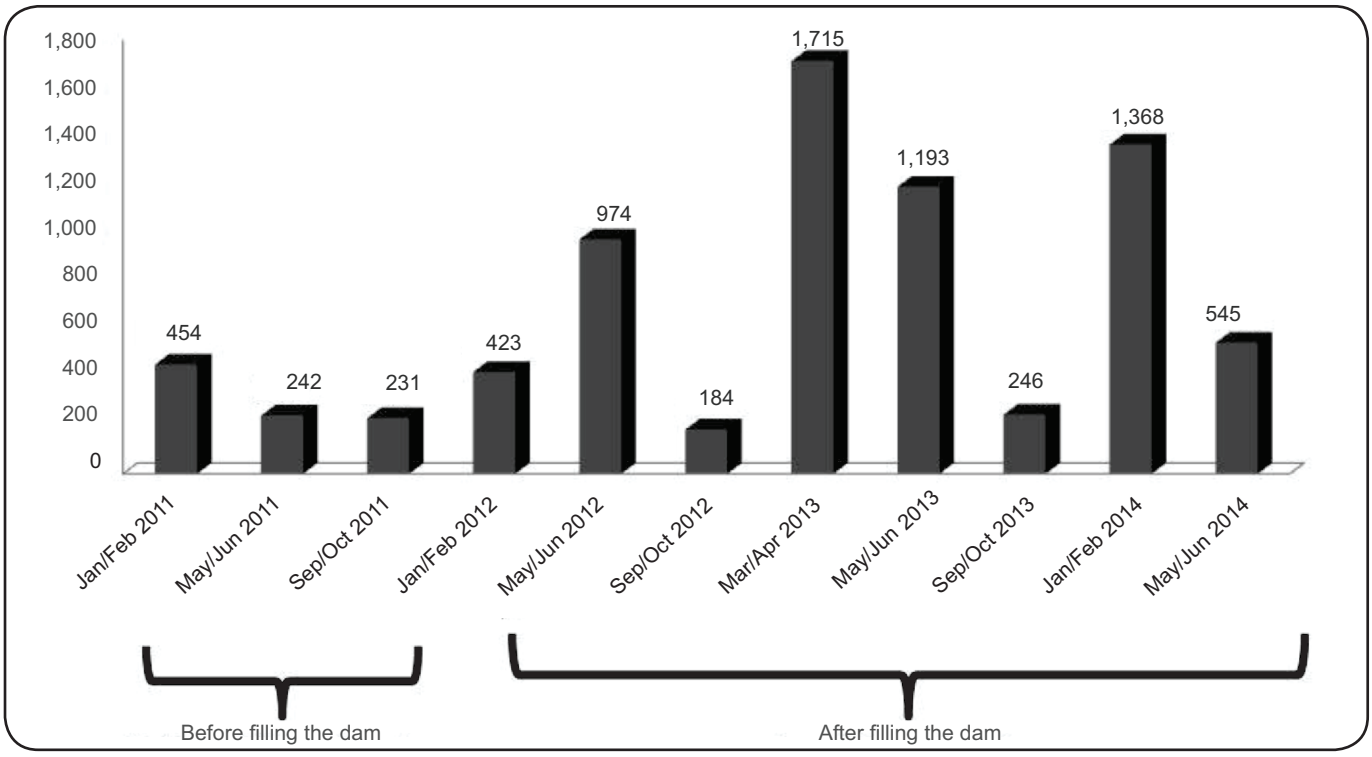

FIGURE 2 - The seasonal variation in number of sand flies collected during the rainy, intermediary, and dry seasons from 2011 to 2014 in the area associated with the Santo Antônio Hydroelectric System.

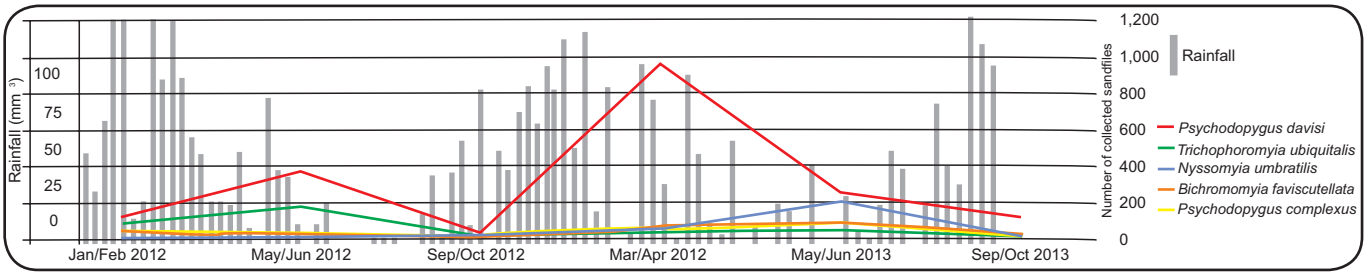

FIGURE 3 - Frequency with which *Psychodopygus davisi, Trichophoromyia ubiquitalis, Nyssomyia umbratilis*, *Bicrhomomyia flaviscutellata,* and *Psychodopygus complexus* were encountered and rainfall during the entomological collections of 2012 and 2013.

observations). These findings and data from the present study further suggest that *Ps. davisi* is involved in the transmission of *Leishmania* (*Viannia*) parasites (mainly *L.* (*V.*) *naiffi*) in the Amazon region[14]. *Trichophoromyia ubiquitalis*, *Nyssomyia umbratilis*, *Bichromomyia flaviscutellata*, and *Ps. complexus* are also likely involved in the transmission of agents of ACL in Brazilian Amazonia.

Trichophoromyia ubiquitalis is the only sand fly vector associated with the transmission of *L.* (*V.*) *lainsoni*[28], a parasite recently identified in State of Rondônia[8]. However, *Trichophoromyia* species are also thought to be carriers of *L. (V.) lainsoni,* as deoxyribonucleic acid (DNA) of this parasite was found in *Trichophoromyia auraensis* specimens in the Peruvian Amazon[29]. Furthermore, two pooled samples of *Th. ubiquitalis* were recently observed to carry the DNA of an ambiguously identified *Leishmania* (*Viannia*) parasite [most likely *L.* (*V.*) *braziliensis*] in the rural area of Cerrado in the State of Mato Grosso[30].

In the present study, *Th. ubiquitalis*, a known vector of *L.* (*V.*) *lainsoni*, was the second most frequently identified species of sand fly in the study area. These observations are in conflict with the hypothesis that other species more anthropophilic than *Th. ubiquitalis* transmit *L.* (*V.*) *lainsoni*-like parasites in State of Rondônia[8] which was suggested by the fact that *Th. ubiquitalis* was infrequently encountered in entomological studies conducted in the municipality of Monte Negro[14]. Although it has previously been suggested that *Th. ubiquitalis* does not exhibit high levels of anthropophilic behavior under field conditions[28], previous studies have described the intradomiciliar transmission of *L.* (*V.*) *lainsoni* via indoor-collected *Th. ubiquitalis* in an island of the metropolitan area of Belém, State of Pará (unpublished observations). These findings suggest that *Th. ubiquitalis* is a putative vector of *L.* (*V.*) *lainsoni* in State of Rondônia.

Nyssomyia umbratilis has been identified as the main vector of *L.* (*V.*) *guyanensis* in northern Brazil, particularly in the Guianan ecoregion complex[31]. The findings of the present study suggest that *Ny. umbratilis* is a frequently encountered species in the Porto Velho municipality, which is consistent with findings of Azevedo et al[13]. These authors found that *Ny. umbratilis* was the second most (19.9%) prevalent species in the Samuel Ecological Station, accounting for 48.5% of the specimens collected in the canopy at 20m. Although the distribution of *L.* (*V.*) *guyanensis* near the southern Amazon River remains unknown, recent findings related to the circulation of a *L.* (*V.*) *guyanensis*-like parasite in State of Rondônia[8] suggest that there is a transmission cycle for this parasite/vector in the studied region. However, additional observations are needed to confirm this hypothesis.

Bichromomyia flaviscutellata[2] [7], the major vector of *L.* (*L.*) *amazonensis,* was frequently encountered in the present survey. This was surprising because light traps were used for collection, and these species typically cannot be collected in large numbers without the use of rodent-baited traps[32]. This unexpected result could be explained by the fact that the traps were placed 50cm above the ground at each CDC collection station where low-flying specimens may have been attracted to the light and intercepted.

Bichromomyia olmeca nociva (Young & Arias), a suspected vector[33] closely related to *Bichromomyia* species which are associated with the epidemiology of *L. (L.) amazonensis,* was detected in this study. This species is similar to *Bi. reducta* (Feliciangeli, Ramirez Pérez & Ramirez), a species that was not observed in the present survey but has previously been described as a natural vector of *L. (L.) amazonensis* in studies conducted in Cachoeira Samuel, State of Rondônia[12]. Because *Bi. flaviscutellata* was detected most frequently in the present study, this species is suspected to be the main species involved in the transmission of ACL attributed to *L.* (*V.*) *amazonensis* in Rondônia[7].

Psychodopygus complexus is currently recognized to be associated with *L.* (*V.*) *braziliensis*-associated ACL in areas where the closely related species *Psychodopygus wellcomei* is absent[34]. In the present study, *Ps. complexus* was unambiguously identified in the collection because of the absence of *Ps. wellcomei* males. However, the transmission of *L.* (*V.*) *braziliensis* by *Ps. complexus* cannot be assumed because no reports of natural infection with this species or evidence of ecological association between the presence of this species and ACL have been reported in the study region. *L.* (*V.*) *braziliensis* is widely distributed throughout Brazil, and several species of sand fly that were not encountered in this study, including *Ps. wellcomei* Fraiha, Shaw & Lainson*, Migonemyia migonei* (França)*, Nyssomyia whitmani* (Antunes & Coutinho), and *Ny. intermedia* (Lutz & Neiva)[2] [7]*,* have been implicated in the transmission of these agents.

The epidemiological background of the highly anthropophilic species *Ps. davisi* should be considered in the context of the transmission of various *L.* (*Viannia*) species in areas of the Amazon region such as Rondônia[14]. The evidence encountered in the present study cannot exclude the possibility that this particular species of sand fly was involved in human infections recently attributed to *L. (V.) braziliensis*[8] in Rondônia.

The area associated with the Santo Antônio hydroelectric system currently has the highest recorded diversity of Phlebotominae in State of Rondônia. The seasonal fluctuations in sand fly populations from the studied region have been shown to be associated with the end of the rainy period, which is consistent with findings from other entomological studies. However, changes in the populations of these insects are also influenced by the filling of the dam. The increased number of sand flies was not found to be associated with an increase in the incidence of ACL.

Psychodopygus davisi, *Trichophoromyia ubiquitalis, Nyssomyia umbratilis*, *Bichromomyia flaviscutellata*, and *Ps. complexus* were the five most frequently collected species in this study, and they are all considered to be potential vectors of ACL agents in the Amazon region. The large number of *Ps. davisi* specimens collected in the present study suggests a need for the entomological surveillance of this sand fly species, as these insects are potential vectors of zoonotic cutaneous leishmaniasis in Rondônia.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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